PROBIOTIC BEVERAGE FROM FLUTED PUMPKIN LEAF JUICE FERMENTED WITH *Pediococcus pentosaceus* IO1

Isaac Ayodeji **Adesina**[1]*, Yinka Doris **Oluwafemi**[1], Victoria Temitope **Adebote**[2]
[1]Department of Biological Sciences, University of Medical Sciences, Ondo City, Nigeria
[2]Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Nigeria
E-mail: iadesina@unimed.edu.ng

Abstract
Recently, there has been renewed interest in the development of non-dairy probiotic beverages. Probiotic cultures are traditionally added to dairy products; however, vegetable juices may represent a suitable mean of delivering these cultures to humans. The aim of this study was to determine the suitability of fluted pumpkin leaf juice as a raw material for the production of probiotic beverage with Pediococcus pentosaceus IO1. Pasteurized pumpkin leaf juice was inoculated with P. pentosaceus IO1 and incubated at 30^{o}C for 48 h. The pH, sugar content, mineral content, and viable cell counts during the fermentation were evaluated. Pediococcus pentosaceus IO1 reduced the pH of pumpkin leaf juice from an initial value of 5.9 to 4.6 after 48 h of fermentation at 30^{o}C; while the viable cell counts of P. pentosaceus IO1 increased from an initial cell concentration of 1.10 x 10^5 CFU/ml (5.04 log CFU/ml) to 1.24 x 10^8 CFU/ml (8.09 log CFU/ml). A slight reduction in the sugar content was observed in the fermented pumpkin leaf juice when compared with unfermented pumpkin leaf juice (control). Fermented pumpkin leaf juice had higher contents of some minerals (Calcium Ca, Magnesium Mg, Iron Fe) and vitamin C than had unfermented pumpkin leaf juice. The fermented pumpkin leaf juice with P. pentosaceus IO1 is a good alternative functional food containing probiotics and it could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

Keywords: Pumpkin leaf juice, probiotic, *Pediococcus pentosaceus*, fermentation

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1. INTRODUCTION

According to Gilliland (2003), probiotics are defined as selected viable microorganisms that, following consumption in a food or feed, have the potential for improving health or nutrition of man or animal. Bacteria in this group may be used to ferment food or are added to food as dietary supplements. Foods for human consumption containing these organisms are sometimes referred to as functional foods (Gilliland, 2003). One of the most significant groups of probiotic organisms are lactic acid bacteria. Lactic acid bacteria constitute a broad heterogeneous group of generally food-grade microorganisms historically used in food preservation and fermentation (Mozzi, 2016).
Probiotic foods are reported to provide several health benefits, as they help in maintaining a good balance and composition of intestinal flora, and increase the resistance against the invasion of pathogens. The demand for probiotic functional foods is growing rapidly due to increased awareness of consumers about the impact of food on health (Tripathi and Giri, 2014). Dairy-based fermented products and yoghurts have been utilized as potential probiotic products since ancient times. The increased popularity of vegetarianism, lactose intolerance, and the high cholesterol content in dairy products are all factors that have recently increased the demand for non-dairy probiotic products (Nematollahi *et al*., 2016).
There has been an increased interest in the development of vegetable juices as functional beverages with probiotics. Vegetables are rich sources of the biologically active compounds which have beneficial effects in the prevention of some diseases and certain types of cancer (Rakin *et al*., 2007; Nematollahi *et al*., 2016).
Fluted pumpkin (*Telfairia occidentalis*) is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. *Telfairia occidentalis* belongs to the Cucurbitaceae

family and is more popular in the South-Eastern part of Nigeria (Akwaowo *et al*., 2000). Fluted pumpkin is widely cultivated for its palatable and nutritive value than other tropical vegetables (Eseyin *et al*., 2014). Previous studies have reported that fluted pumpkin leaves are rich in minerals (such as iron, potassium, sodium, phosphorus, calcium, and magnesium), vitamins (such as thiamine, riboflavin and ascorbic acid), antioxidants, and phytochemicals (Akwaowo *et al*., 2000; Oboh, 2005; Kayode and Kayode, 2010; Eseyin *et al*., 2014).
The objective of this study was to determine the suitability of fluted pumpkin leaf juice as a raw material for the production of probiotic beverage with *Pediococcus pentosaceus* IO1.

2. MATERIALS AND METHODS

2.1 Preparation of Pumpkin Leaf Juice

Pumpkin leaves (*Telfairia occidentalis*) were purchased from a local market and kept at 4^{o}C prior to use. After the pumpkin leaves were washed thoroughly, the juice was extracted and prepared from the leaves using a juice extractor under aseptic condition. The freshly prepared pumpkin juice was pasteurized at 80^{o}C for 5 min.

2.2 Bacterial Strain and Growth Condition

A lactic acid bacterial strain, *Pediococcus pentosaceus* IO1, was used for this study. *Pediococcus pentosaceus* IO1 was grown anaerobically at 30^{o}C for 24 h in de Man Rogosa Sharpe (MRS) broth.

2.3 Fermentation of Pumpkin Leaf Juice

Probiotic pumpkin leaf juice preparation was done by fermentation with *P. pentosaceus* IO1 according to the methodology described by Yoon *et al*. (2006). Pasteurized pumpkin leaf juice was inoculated with 2% of 24 h old inoculum of *P. pentosaceus* IO1 and incubated at 30^{o}C for 48 h. The juice sample without inoculum of *P. pentosaceus* served as the control. Samples were taken at 0, 24, and 48 h for chemical and microbiological analyses.

2.4 Chemical Analyses

The pH of pumpkin leaf juice was measured with a pH meter standardized with buffer solution of 4.0 and 7.0 (AOAC, 2005). Sugar content (total soluble solid) expressed as ^{o}Brix was determined using a refractometer (Sanchez-Moreno *et al*., 2003). Vitamin C (Ascorbic acid) content of the juice was determined by the method of AOAC (2005). Minerals (calcium, magnesium, and iron) were determined using atomic absorption spectrophotometer (AOAC, 2005). Sodium and potassium contents were determined using the flame photometric method (AOAC, 2005).

2.5 Microbiological Analysis

Viable cell counts (log CFU/ml) of lactic acid bacterial culture were determined by the standard plate method with MRS agar after 48 h of incubation at 30^{o}C.

2.6 Statistical Analysis

All experiments were carried out in duplicate. The results are expressed as mean ± S. D. (standard deviation). The SPSS software package for MS Windows version 16 was used to analyze the experimental data.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of the Fluted Pumpkin Leaf Juice During Fermentation

The result of pH revealed that there was a reduction in pH from an initial value of 5.9 in unfermented pumpkin leaf juice (control) to a pH of 4.6 in juice treated with *Pediococcus pentosaceus* IO1 after 48 h of fermentation (Figure 1). The pH value of pumpkin leaf juice dropped from 5.9 to 4.6 due to the production of acidic metabolites such as lactic acid by *Pediococcus pentosaceus* IO1. Due to acid production, most lactic acid bacterial strains are able to inhibit the growth of undesirable accompanying microorganisms in fermentation medium. It is well known that lactic acid increases the nutritional value of fermented bioproducts by engendering better taste and structure of the fermented bioproducts (Rakin *et al*., 2007; Kun *et al*., 2008). Similarly, Yoon *et al*. (2006) reported a reduction in pH value of cabbage juice by three species of lactic acid bacteria (*Lactobacillus casei*, *L. plantarum*, and *L. delbrueckii*) during 72 h of fermentation.

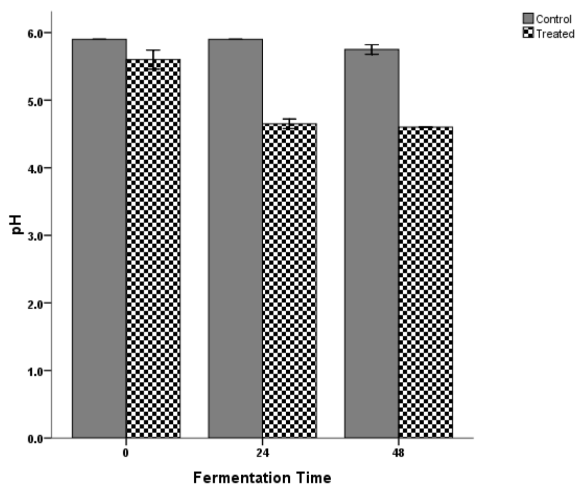

Fig. 1: pH value of pumpkin leaf juice (control) and juice treated with *P. pentosaceus* IO1 during fermentation

The content of sugar in pumpkin leaf juice at the end of fermentation is shown in Figure 2.

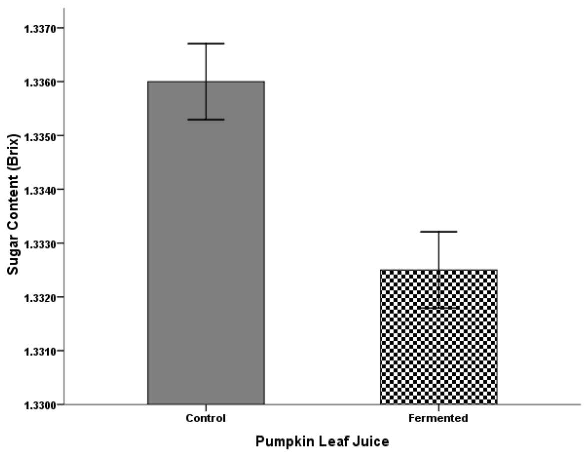

Fig. 2: Sugar content in pumpkin leaf juice after 48 h of fermentation

The result shows that the content of sugar in unfermented juice (control) was higher than the fermented juice. This result suggests that some of the soluble sugars may have been utilized by *P. pentosaceus* IO1 for its growth, and a part of the soluble sugar may also have been converted to lactic acid which increases the acidity of the fermented juice (Rodrigues *et al*., 2012). Rodrigues *et al*. (2012) reported that total sugars in probiotic orange and peach juices decreased during fermentation and storage, which was attributed to the growth of probiotic culture used and sugar fermentation. These results reflect those of Panda *et al*. (2017) who also found a reduction in the total sugar content and total soluble solid of prickly pears juice during probiotic fermentation.

The content of vitamin C in pumpkin leaf juice after 48 h of fermentation is shown in Figure 3. Vitamin C content was higher in fermented juice with *P. pentosaceus* IO1 than the unfermented juice (control). A slight increase in vitamin C content from 21.49 mg/100g to 22.3 mg/ 100g was observed in the fermented pumpkin leaf juice after 48 h of fermentation. This finding is contrary to that of Panda *et al*. (2017) who observed a significant reduction in the ascorbic acid (vitamin C) content of Prickly pears juice during fermentation. The increase in the vitamin C (ascorbic acid) content of the fermented pumpkin leaf juice observed in this study is desired not only from the nutritional point of view, but also due to the fact that it could promote anaerobic conditions acting as an oxygen scavenger (Shah *et al*., 2010; Buruleanu *et al*., 2013).

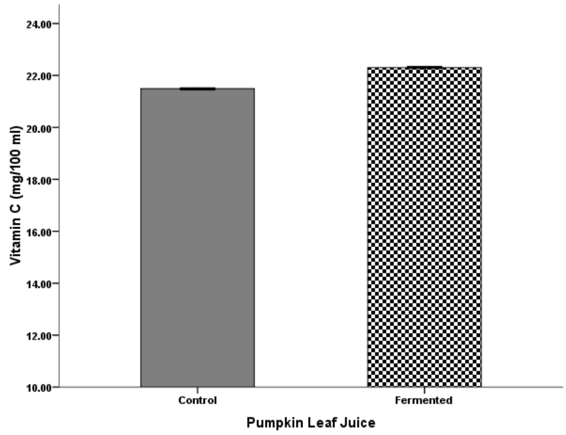

Fig. 3: Vitamin C content in pumpkin leaf juice after 48 h of fermentation

Vitamin C is the major water-soluble antioxidant and acts as a free radical scavenger. The regular intake of fresh vegetables, containing vitamin C along with many other

vitamins and micronutrients, can reduce the incidence of various cancers (Kakizoe, 2003).
The contents of minerals in fermented pumpkin leaf juice are presented in Table 1. The result shows that the fermented pumpkin leaf juice had higher contents of some minerals (calcium, magnesium, and iron) than had unfermented pumpkin leaf juice (control).

Table 1: Mineral contents (mg/100 ml) in pumpkin leaf juice after 48 h of fermentation

Mineral contents	Control	Fermented Juice
Calcium (Ca)	58.00^{a}±1.41	63.00^{a}±1.40
Magnesium (Mg)	87.00^{b}±0.10	94.00^{a}±0.10
Potassium (K)	406.00^{a}±2.83	396.00^{b}±1.41
Sodium (Na)	18.00^{a}±1.41	16.00^{a}±2.00
Iron (Fe)	1.00^{a}±0.02	1.40^{a}±0.01

A possible explanation for this might be that *P. pentosaceus* IO1 strain utilized a small part of these minerals for its growth. Increase in some of the mineral contents confers additional nutritional benefits to this functional beverage from fluted pumpkin leaf juice. Fermented pumpkin leaf juice can be a good source of iron and can therefore alleviate iron deficiency. The high content of iron in the extract of fluted pumpkin leaves has been given as the reason for its administration as blood tonic in the traditional treatment of anemia (Eseyin *et al.*, 2014).

3.2 Probiotic Culture Viability in Fluted Pumpkin Leaf Juice

The changes of viable cell counts of *P. pentosaceus* IO1 during fermentation of pumpkin leaf juice are presented in Figure 4. The viable cell counts of *P. pentosaceus* IO1 increased significantly from an initial cell concentration of 5.04 log CFU/ml to 8.09 log CFU/ml after 48 h of fermentation. *Pediococcus pentosaceus* IO1 was found capable of growing well in pumpkin leaf juice.

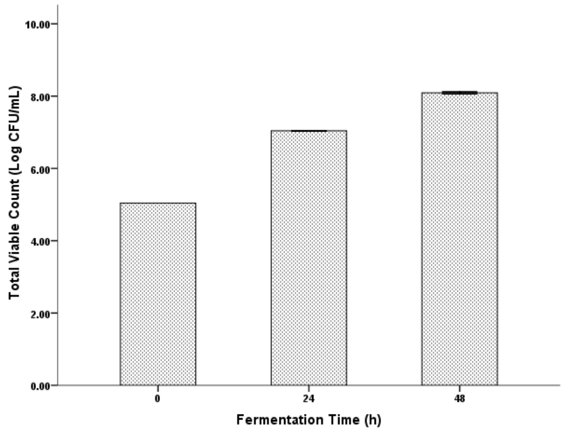

Fig. 4: Viable cell count of *P. pentosaceus* IO1 in inoculated pumpkin leaf juice during fermentation

It could survive the low pH in the fermented pumpkin leaf juice. The low pH influenced the growth of P. pentosaceus IO1 by allowing it to grow rapidly in the juice. Based on these results, pumpkin leaf juice appeared to be very promising as a growth medium for Pediococci. Pereira *et al.* (2013) reported that viable cell counts of *Lactobacillus casei*, used as probiotic bacteria, increased in cashew apple juice during fermentation. Growth capacity of probiotic bacteria is important for the success of fermentation processes and the viability of these bacteria is crucial for the quality and stability of fermented products (Kun *et al.*, 2008). Fermented products containing at least 7.0 log CFU/ml of viable probiotic bacteria at the time of consumption confers beneficial effects on intestinal microflora of the host (Gobbetti *et al.*, 2010).

4. CONCLUSION

Fluted pumpkin leaf juice could be used as a suitable substrate for the growth of *P. pentosaceus* IO1 strain. Based on the results of this study, it is concluded that *Pediococcus pentosaceus* IO1 could be used as a probiotic culture for production of a functional beverage from fluted pumpkin leaf juice. The fermented pumpkin leaf juice could serve as a healthy beverage for vegetarians and consumers who are allergic to lactose present in probiotic dairy products.

5. REFERENCES

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